UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

OFFICE OF PESTICIDE PROGRAMS
REGISTRATION DIVISION (7505P)

March 9, 2012

MEMORANDUM: Response to Intervet's Rebuttal dated January 18, 2012 (Companion Animal Safety)

Subject:	Name of Pesticide Product:	ACTIVYL TICK PLUS
	EPA Reg. No. /File Symbol:	773-95
	DP Barcode:	DP 399051
	Decision No.:	452521
	Action Code:	R340
	PC Codes:	109701 (Permethrin: 42.50%)
		067710 (Indoxacarb: 13.01%)

From: Byron T. Backus, Ph.D., Toxicologist
Technical Review Branch
Registration Division (7505P)

Byron T. Backus March 9, 2012
M Hasbrouck Team Leader/Toxicology

To: Autumn Metzger/Venus Eagle RM 01
Insecticide-Rodenticide Branch
Registration Division (7505P)

Registrant: INTERVET INC.

FORMULATION FROM LABEL:

Active Ingredient(s):	by wt.
067710 Indoxacarb	13.01%
109701 Permethrin	42.50%
Other Ingredient(s):	44.49%
TOTAL	100.00%

ACTION REQUESTED: The Risk Manager requests:

"This is Intervet/Schering/Merck's formal rebuttal to your memo dated November 30, 2011, which denied their request for "normal" dosing due to adverse effects… Please see enclosed package with cover letter explaining what happened and their rebuttal (MRID 48721701)… In addition, in their

original submission they had requested breeding claims but were also denied. I am not sure if they are rebutting that or not…"

BACKGROUND:

The material received for review includes a cover letter (dated January 18, 2012) from the registrant and a 62-page document (MRID 48721701) titled: "ACTIVYL Tick Plus for Dogs and Puppies: Companion Animal Safety Rebuttal."

COMMENTS AND RECOMMENDATIONS:

1. The following is from pages 728-729 of the original report in MRID 48555502:

Endpoint	Gender	Group	Covariate Mean	Statistics	Day 71
Absolute Reticulocytes, $10^3/\mu L$	Pooled	Control	88.47	Mean	66.94
				SD	25.083
		1X	108.33	Mean	89.39
				SD	27.728
				p-value	0.1577
		3X	97.68	Mean	115.86
				SD	66.519
				p-value	0.0016*
		5X	97.15	Mean	161.63
				SD	61.391
				p-value	0.0000*

From the TRB review of November 30, 2011:

On day 71, 3X males and 5X animals of both sexes had reticulocytosis with group mean percent reticulocyte and absolute reticulocyte values that fell outside the provided reference ranges: in ascending group order, mean percent reticulocytes were 1.33%, 1.63%, 2.67%, and 2.83% in males and 0.82%, 1.30%, 1.30%, and 2.57% in females (vs. normal values of ≤1.8% in both sexes).

Based on the occurrence of treatment-related reticulocytosis at the 3X dose level in males, it is concluded that the margin of safety in puppies treated three times at 28-day intervals with Indoxacarb/Permethrin (SCH 900560) Topical Solution is 1X the recommended dose, which is less than the 5X margin of safety indicated in the 870.7200 Companion Animal Safety Guidelines.

2. The initial part of the rebuttal in MRID 48721701 focuses on the statistical relevance of the reticulocyte counts of the study in MRID 48555502. From p. 7 of MRID 48721701: "…This numerical (statistical) significance does not definitely define the change or trend as biologically real or biologically relevant, which can only be established with the consideration of the associated pathophysiology. Simply put, this numerical issue that occurred with mean reticulocyte values, for both the percent and absolute counts, constitutes a chance sampling error and does not

indicate a toxicologically significant finding. As will be discussed in the following sections, the reticulocyte values observed in the 3X and 5X groups in the 8-week I/P puppy safety study **(S10048-00; MRID 48555502)** are not toxicologically significantly increased, as they are within the reference ranges from various sources and not biologically relevant."

3. Reticulocytosis has been observed in rats and dogs following subchronic and chronic feeding of indoxacarb. For example, from TXR 0013557:

   In a subchronic toxicity study (MRID 44477131), DPX-JW062-106 (50% DPX-KN128, 50% DPX-KN127; 95.03% a.i.; lot number not given) was offered in feed to 4 beagle dogs per sex at dose levels of 0, 40, 80, 160, or 640 ppm for 13 weeks. The average measured doses for the corresponding groups were 0, 1.0, 2.5, 5.0, and 19.2 mg/kg (body weight)/day for males, and 0, 1.1, 3.1, 5.0, and 18.8 mg/kg/day for females. All animals survived to study termination. Compound administration was not related to changes in body weights, body weight gains, food consumption or feed efficiency. Various hematologic parameters were affected in all dosage groups, although definitive dose responses were not always observed. Throughout the study, the 640-ppm males and females had the lowest for hemoglobin and MCHC values, and the highest MCV values. The 640-ppm females had the lowest RBCs and highest platelet and reticulocyte counts among the treated females. Although intragroup temporal trends were not observed for absolute values, when expressed as percentages of control values, reticulocytes increased over time in the high-dose groups (both sexes), hemoglobin and MCHC values decreased over time in high-dose females, and platelets increased in the 160- and 640-ppm females. Hemolytic anemia in the 160-ppm males and in the 640-ppm females was identified by statistically ($p \leq 0.01$-0.05) and biologically significant decreases in hemoglobin (78.0-87.3% of control values) and RBCs (76.3-84.8% of control values). High-dose animals had platelet and reticulocyte counts ranging from 145.2-164.4% and 566.7-2300.0% of control values, respectively. At the low dose of 40 ppm (both sexes) there were decreases in hematocrit (92%of control values), hemoglobin (88-91% of control values) and RBCs (85-87% of control values) by 3 weeks with compensation evident by 8 weeks. This compensation was not present in the 160 and 640 ppm groups. The increased platelets indicates, that although there was compensation of some parameters, the hematopoietic effects were ongoing throughout the treatment period. Microscopic findings considered secondary effects of a compound-related hemolysis included increased pigment (interpreted as hemosiderin and other blood breakdown products) in Kupffer cells, renal tubular epithelium, and spleen and bone marrow macrophages; minimal to mild increases in extramedullary hematopoiesis in the spleen; and minimal to mild erythrocytic hyperplasia in bone marrow. Except for the increased macrophage pigment and extramedullary hematopoiesis in the spleens of treated females, the findings exhibited dose-response relationships. Findings were observed in one or more animals from each treated group, with the exception of increased extramedullary hematopoiesis, and increased macrophage hemosiderin and erythrocytic hyperplasia in the bone marrow (no occurrence in 40-ppm males), and increased renal tubular epithelial cell pigment (no occurrence in 40-ppm females). **Under the conditions of this study the systemic LOAEL is 40 ppm (1.0 mg/kg/day for males and 1.1 mg/kg/day for females) based on biologically adverse hemolytic anemia, as indicated by decreases in hemoglobin, hematocrit, RBCs; increases in platelets; and secondary histopathologic findings indicative of blood breakdown (pigment in Kupffer cells, renal tubular epithelium, and spleen and bone marrow macrophages; increases in splenic extramedullary hematopoiesis; and erythrocytic hyperplasia in bone marrow. A NOAEL was not established for males and females.**

4. TRB concludes that the dose-related increase in reticulocytes observed in the puppy study may have been caused by exposure to indoxacarb. However, as noted by the registrant (p. 10 of MRID 48721701): "…there were no associated changes in other hematology parameters (*e.g.* decreased hematocrit, erythrocyte count and hemoglobin) that one would expect if the higher reticulocytes values were a real effect." Also (from p. 11): "the Agency has already agreed that there were no significant decrease[s] in hematocrit, erythrocyte count, or hemoglobin. Therefore, there was no evidence for hemolytic anemia or other blood loss in this study." In addition: "the Agency stated that the higher reticulocyte values were considered to be an adverse effect because they could indicate some underlying disease processes such as hypoxia causing stimulation of the bone marrow to release reticulocytes. …hypoxia…can occur by two mechanisms: 1) inadequate oxygen carrying capacity of the blood (due to anemia or altered hemoglobin function); or, 2) inadequate tissue perfusion. None of the puppies exhibited lethargy , panting or cyanosis of the mucous membrane, commonly seen clinical signs associated with hypoxia. As previously discussed, there was no anemia noted in this study. In addition, there was no Heinz body induction and there were no observations of brown-discolored blood, indicative of oxidative damage and methemoglobin formation." Overall then the slightly increased and dose-related increase in reticulocytes was not toxicologically significant.

5. The registrant's rebuttal in MRID 48721701 includes a section (starting p. 13) on margin of safety and dose multiples, noting that: "The exact mg/kg doses were maintained throughout the 8-week I/P puppy study…even though the puppies were rapidly growing and thus had increasing body weights. The 8-week I/P puppy study…was conducted to support the use of the 0.5 mL applicator in puppies…each applicator contains approximately 75 mg of indoxacarb and 240 mg of permethrin… The smallest puppy weight listed on the label is 4 lb (1.8 kg) body weight. Thus, the highest possible mg/kg dose that a puppy would be exposed to by the 0.5 mL applicator is approximately 42 mg of indoxacarb and 133 mg of permethrin per kg body weight. (75 mg indoxacarb / 1.8 kg = 41.67 mg of indoxacarb/kg BW; 240 mg permethrin / 1.8 kg BW = 133.3 mg permethrin/kg BW)…

   "Because the young puppies gain body weight rapidly, the actual mg/kg dose administered by a 0.5 mL applicator will rapidly decrease over time as the puppies grow. For example, based on the growth data from the 8-week I/P puppy safety study…and the proposed label, when an 8-week old puppy weighing 4 lb (1.8 kg) is dosed with one 0.5 mL applicator it will receive the maximum possible mg/kg dose of 42/133-I/P mg/kg. However, one month later, the now 12-week old puppy will probably weigh about 8 lb (4.1 kg), so the mg/kg dose is now about 18/59-I/P mg/kg (75 mg/4.1 kg = 18.3 mg/kg and 240 mg/4.1 kg = 58.5 mg/kg). Then a month later, the 16-week old puppy will probably weigh 12 lbs (5.5 kg), so the mg/kg dose from a 0.5 mL applicator would be about 14/44-I/P mg/kg (75 mg/5.5 kg = 13.6 mg/kg and 240 mg/5.5 kg = 43.6 mg/kg)…

   "It is important to note that the rigorous exact mg/kg dosing multiple regimens used in the 8-week I/P puppy safety study…did not allow for dose reduction due to growth (which would have resulted in lower mg/kg doses). Instead, the exact mg/kg dose multiples continued to be administered throughout the study… In clinical veterinary practice, puppies would be dosed using the 0.5 mL applicator; they are not dosed on a mg/kg basis…in order to evaluate the safety study data for the margin of safety, the actual mg/kg administered doses in the 8-week I/P puppy safety study…need to be converted to the equivalent number of 0.5 mL applicators that would be required to be administered to deliver that specific mg/kg dose."

Using this argument, the registrant has calculated (see p. 16 of MRID 48721701) the treatment-related effects of decreased food consumption and body weights in Group 4 puppies in the second half of the dosing period to high-dose Applicator multiples (9.4-13.4 Applicator dose equivalents). No adverse food consumption or body weight decreases were noted in Group 4 puppies following the initial application on day 1 (up to 9 Applicator doses).

Although not mentioned by the registrant, there are also changes in the body surface-to-volume ratio. As the animal grows, the surface-to-volume ratio decreases; if dermal dosing is based on body weight (which is proportional to volume) then the effect would be an increased application rate over the animal's surface area. Another way of looking at the situation would be in terms of dosage to the animal's nervous system; the nervous system does not grow at the same proportional rate as the rest of the body, so if dosage is kept constant in terms of body weight while the animal is developing it would actually increase relative to the weight of the animal's nervous system.

6. From the original TRB review dated November 30, 2011: "Mean food consumption of 5X males was decreased throughout the entire study (12-40% less than controls), which resulted in a 22% reduction in this group's mean overall food consumption. This correlated with decreased mean cumulative weight gain in the 5X male group (19% less than controls), with absolute body weight also affected, measuring approximately 11%-15% less than controls from day 32 through the end of the study." The following table shows the mean weight gains for male puppies in the different dosage groups for the periods of days 1 to 29 and 29 to 57:

Mean Weight gains for male puppies (kg) ± S.D.[a]				
Interval	Dosage			
	Control	1X	3X	5X
Males				
Days 1 to 29	1.948 ± 0.315	1.585 ± 0.185	1.632 ± 0.454	1.473 ± 0.282
Days 29 to 57	1.258 ± 0.350	1.617 ± 0.361	1.500 ± 0.217	1.092 ± 0.353

[a] Calculated from data on pp. 26-29 of MRID 48721701.

The data show that the 5X male puppies had a lower mean weight gain relative to controls in the period from day 1 to 29, but the value was comparable to those for the 1X and 3X groups. However, the mean weight gain for the 5X male puppies was considerably less than the other groups in the period from day 29 to 57.

From p. 15 of the registrant's rebuttal in MRID 48721701: "In the 8-week I/P puppy safety study…the final study report describes that there were treatment-related decreased food consumption and body weight gains in the Group 4 (5X) males starting around Study Day 28 to 35. …Group 4 males were dosed on Day 29 with dose volumes ranging from 4.7 to 6.7 mL, which corresponds to 9.4 to 13.4 applicators (0.5 mL) since all puppies weighed below 5 kg. Thus, the treatment-related decreased food consumption and body weights observed in Group 4 were noted after 9.4 to 13.4 Applicator-Dose multiples were administered. In contrast, the initial (Day 1) dose volumes in Group 4 males ranged from 3.0 to 4.5 mL, which correspond to 6 to 9 Applicator-Dose multiples. It is important to note that Group 4 puppies did not show decreased food consumptions or body weights after the initial dosing. Therefore, this dosing data shows that Applicator-Dose multiples up to 9X were well tolerated in Group 4 puppies. This strongly

indicates that the 8-week I/P puppy safety study…demonstrated at least a 5X Margin of Safety for the treatment-related decreased food consumption and body weights.

TRB accepts the registrant's argument that the reduced weight gain in Group 4 males in the period from day 29 to day 57 was associated with dosage rates >5X.

7. From p. 18 of the TRB review dated November 30, 2011: "…In this study, the 3X and 5X doses were accomplished by either extended duration of application, extension of the application site caudally along the dorsal midline or repeated passes over the dorsal midline. According to the proposed product label, the product is supposed to be applied as a single dose at the base of the neck for animals in this age group. Thus, the product was not applied as proposed. In addition, the volume of solution applied to each animal was not reported.

   From page 17 of the registrant's rebuttal in MRID 48721701: "The test material was applied as per the study protocol and approved label directions (between the shoulder blades), and then as needed along the dorsal midline. In addition, it is not critical that this product be placed in [a] single application site since the approved product label for larger breed dogs (thus with larger dose volumes) allows for the application at multiple sites along the dorsal midline… The previous pilot 2-week study…has shown that application of such large volumes of test material resulted in marked migration. Such migration of the test materials to the area of the shoulder blades or along the sides of the animal would increase the likelihood that material could drip off of the puppy, which would result in under-dosing that animal. Thus, for the 8-week I/P puppy safety study… the duration of dose application for each puppy was extended, so that the test article could be applied more slowly in order to try and minimize migration down the neck and sides of the animals…

   "In addition, the Agency commented that the dose volumes applied to each animal on each dosing day were not included in the final study report… The dose volumes were compiled from the computer data files by the testing facility and are provided in Appendix B [p. 26-29 of MRID 48721701]."

8. From p. 18 of the TRB review dated November 30, 2011: "The animals were given routine concurrent treatments during the study, including vaccinations on days 2 and 24 (or 3 and 25), deworming with Albon® on days -8 through 4 (or -7 through 5), and deworming with pyrantel pamoate on days 2 and 24 (or 3 and 25)."

   From p. 18 of the registrant's rebuttal in MRID 48721701:

   "Because the indoxacarb dosing was to begin when animals were 8 weeks-old, there was not sufficient time in the acclimation period to complete all of the routine vaccinations and deworming/coccidial treatments required by puppiesprior to the initiation of dosing. Label instructions for vaccinations require an initial vaccination and 2 boosters 3-4 weeks apart and therefore could only be accomplished by administration during both the acclimation and experimental periods. Also, the vaccination schedule should not be initiated at an earlier age since maternal antibodies that the puppies receive will negate the value of vaccinating the animals. Similarly, parasite evaluations could only take place after animals had been weaned and were individually housed. Therefore, parasite evaluation and any treatment needed to ensure animals

were free of infectious diseases would have to take place during both acclimation andexperimental phases.

"The use of appropriate vaccinations and medications are required when dealing with such young animals. It is unlikely that the testing facility Animal Care and Use Committee would ever approve a protocol if such vaccinations and medications were not allowed in juvenile animal studies.

"Also, the 8-week I/P puppy safety study (S10048-00; MRID 48555502) was done under the "worst-case" scenario, allowing use of vaccinations and concurrent medications. If there had been some sort of adverse interaction with the anticoccidial medication, this would indicate a potential safety issue. However, no such effects were observed in the study. Thus, Activyl Tick Plus can be safely used on young puppies, even if they are being treated for coccidia or vaccinated."

9. From p. 18 of the TRB review dated November 30, 2011: "The Guideline states that hematology and clinical chemistry testing should be done prior to treatment and 24 hours post-treatment. In the current study, the puppies were treated on Days 1, 29 and 57, and clinical pathology testing was done on Days -5, 30, 58, 64 and 71. No testing was done 24 hours after the first treatment.

   From p. 19 of the registrant's rebuttal in MRID 48721701:

   "Clinical pathology was not performed 24 hours after 1st treatment (Study Day 1) because these small young puppies had blood collected from them on Study Day -5 and needed time to recover from the decrease in their blood volume. The testing facility Animal Care and Use Committee did not approve of blood sample collection within 2 weeks from such young small animals. Additionally, any treatment-related effects on clinical pathology parameters after the 1st treatment would likely be identified from the same analyses after the 2nd and 3rd treatments and would therefore be sufficient in assessing effects on these parameters. In addition, for the pilot 2-week I/P puppy study (S10045-00; MRID 48555501), the puppies were older (14-15 weeks) and blood samples were collected for hematology 24 hours after dosing. These puppies were all over 5 kg at the time of dosing, and thus were within the weight range for the 1-mL applicator. These puppies were dosed with volumes of 4.7 - 8.3 mL which correspond to approximately 5 - 8X Applicator-Doses. Despite these high dose multiples, the pilot 2-week study did not show any treatment-related hematology changes at 24 hours after dosing."

10. From p. 18 of the TRB review of November 30, 2011: "The 870.7200 Companion Animal Safety Guidelines state that routine sacrifice or necropsy is not required for surviving animals. In the current study, all of the puppies were sacrificed at the end of the study."

    From p. 19 of the registrant's rebuttal in MRID 48721701:

    "It is regrettable that normal healthy animals had to be euthanized at the end of the study. However, this was a GLP-compliant study and testing facility, and GLP studies require the use of naïve animals. Therefore, these animals could not be reused on any future GLP study."

11. From p. 18 of the TRB review of November 30, 2011: "Specific examinations of the application site should have been conducted."

From p. 19-20 of the registrant's rebuttal in MRID 48721701:

"The topical application site is on the skin, and therefore was evaluated as part of the skin/integument evaluation during the twice daily detailed clinical observations and the physical examinations by personnel that were blinded to treatment group information. Review of the clinical observation group summary data tables indicate various findings (e.g., sparse hair, nodules, red discolorations or thickened skin), but there are no dose-dependent trends. Although thickened skin was frequently noted in one 5X female (#932) the lesion was on the right ear, not the application site. Cranial sparse hair was frequently noted in some puppies, but was almost exclusively in the saline control males and a IX male and a 3X female, but not in any 5X group animals. The sporadic skin nodules and red discolorations were either periocular or on the ears in either saline control or 3X animals. Therefore, there were no test article-related application site changes observed in the study."

12. From p. 18 of the TRB review of November 30, 2011: "Housing two animals per cage through day 28 complicated the assessment of the clinical observations and food consumption data. For example, in the statistical analyses, it was assumed that each puppy ate an equal amount during this time period, and this may not have been the case. Also, the food consumption data through day 28 did not include any canned or moistened diet that was offered."

    From p. 19-20 of the registrant's rebuttal in MRID 48721701:

    "It is important to note that when one is conducting young puppy studies that the puppies are quite stressed because they have to adapt to many changes in a very short span of time and in a very critical phase of their lives. For this study, the puppies were weaned at 6 weeks-old and then shipped to the testing facility. The puppies have to adapt to being away from their dams and multiple siblings. They are in a different housing environment with new personnel and need to adapt to dietary changes, all before dosing starts when they are 8 weeks-old. Individual housing of the puppies would only add more stress.

    "A few years ago, MAH did try to do a puppy study with individually-housed puppies, but many of the puppies failed to thrive, and the study was cancelled before dose initiation. A new study had to be started with another set of puppies, but for this new study, the puppies were initially pair-housed. …For the above reasons, we believe pair-housing is a necessary and appropriate husbandry practice for the conduct of young puppy studies, and thus is not a study deficiency."

13. From p. 18 of the TRB review of November 30, 2011: "The narrative summary of physical examin findings stated that animal 912 (a 3X male) exhibited decreased conscious proprioception of the right front foot during the examination on day 31 (p. 187); however, the tabulated summary of cranial nerve and reflex scores indicated that all six 3X males tested normal for right front limb proprioception on day 30 (p. 200), and the abnormality was omitted from this animal's individual data (p. 486)."

    It is noted that the actual comment on p. 187 of MRID 48555502 was: "On neurological examination, animal 912 exhibited decreased conscious proprioception for the right front foot. This could be due to excessive compliance of the animal during the exam. Otherwise, animals 901-924 were neurologically normal." The findings on page 486 are limited to 3X animal 910; the findings for animal 912 are on page 482 and the number following right for day 30 is "1" rather than the usual "2."

From p. 21 of the registrant's rebuttal in MRID 48721701:

"We do not understand this comment. Male # 912 is in the IX group, not the 3X group. Page 187 states that animal #912 had decreased conscious proprioception in the right front foot on Study Day 30, not 31. Since page 200 shows that all of the 3X males had normal forelimb conscious proprioception, that is an accurate statement. Also, Page 198 which is the correct page to show the group summary data for IX males accurately states that one IX male had decreased conscious proprioception in the right forelimb on Day 30. Therefore, we believe that there is no error in the table. In contrast, Page 486 does not mention about animal #912, nor is there any statement on the page about any data being omitted, as indicated in the EPA comment. Therefore, this comment does not show any deficiency in the study report."

14. **TRB concludes that the registrant has adequately addressed the concerns of the TRB review dated November 30, 2011 and that the study in MRID 48555502 indicates a 5X margin of safety in 8-week old puppies dosed at the label rate of 0.5 mL for dogs and puppies weighing 4-11 lbs. While it is possible that the reticulocytosis observed at the 3X and 5X doses levels was an effect of exposure to the test material, it is not considered a toxicologically adverse effect in the absence of changes in other hematology parameters. Effects observed in this study (including reduced weight gain in Group 4 males in the period from day 29 to day 57) were associated with dosage rates >5X. This study supports the use of this product at monthly intervals at the following dosage rates: 0.5 mL for dogs and puppies weighing 4-11 lbs (1.8-5.0 kg); 1.0 mL for dogs and puppies weighing 11-22 lb (5.0 to 10.0 kg); 2.0 mL for dogs 22-44 lbs (10-20 kg); 4.0 mL for dogs 44-88 lb (20-40 kg); and 6.0 mL for dogs 88-132 lb (40-60 kg).**